PHYSIOLOGY.—*Observations on the oxygen consumption of young* Australorbis glabratus. ALINA PERLOWAGORA-SZUMLEWICZ[1] and THEODOR VON BRAND, National Institute of Allergy and Infectious Diseases.[2]

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The oxygen consumption of developing eggs of *Australorbis glabratus* has been studied from the time of oviposition till the hatching period (Perlowagora-Szumlewicz and von Brand, 1957), and several investigations on the respiration of sexually mature specimens of this species have been published (von Brand et al., 1948, 1953; Edwards et al., 1951). However, no detailed investigation has been made of young specimens; that is, for the interval between hatching and reaching sexual maturity. The present study was designed to close this gap in our knowledge.

MATERIAL AND METHODS

Australorbis glabratus (Venezuelan stock, laboratory maintained since 1947) hatched from the eggs used in our previous study (Perlowagora-Szumlewicz and von Brand, 1957) were isolated during the first 24 hours after hatching into jars containing dechlorinated water and lettuce leaves. Thus a supply of snails of rigorously controlled age was available. The very young snails (freshly hatched to 1 week old) were fairly sensitive to handling, especially during the drying procedure preliminary to weighing. These snails were used therefore only for a single oxygen and weight determination and were then discarded. All other snails, beginning 18 to 21 days after hatching, withstood the necessary manipulations remarkably well and therefore were retested at weekly intervals. The groups established for initial testing were kept separately in beakers between tests and were fed abundantly with lettuce. Powdered calcium carbonate was added at weekly intervals. With increasing size of the specimens, subdivision into smaller groups became necessary.

The oxygen consumption was studied by means of the standard Warburg technique at a temperature of 28°C. Flasks of 5-ml capacity with 1 ml dechlorinated water as medium and flasks of 16 ml capacity with 3 ml water were used, depending on the size of the snails. The experimental period lasted 2 to 3 hours; readings were taken every half hour. The number of specimens per flask varied, depending upon their size. Up to 150 of the smallest and only 1 or 2 of the largest were used. The former were counted under a dissecting microscope prior to the introduction into the Warburg flask which was done with the help of a very fine spatula and a brush. At the end of the experiment the snails were washed out of the flask onto filter paper. They were shifted once or twice to fresh filter paper and allowed to dry for 20 to 30 minutes in air before being weighed on a microbalance to the nearest 0.001 mg. The older snails were dried according to Newton and von Brand (1955) and weighed to the nearest 0.1 mg.

RESULTS AND DISCUSSION

Biological observations.—Because little is known about postembryonic growth of *Australorbis glabratus*, we are describing some of our relevant observations.

Upon plotting the weight of snails as function of age on a double logarithmic scale, two bisecting lines resulted, indicating that the growth characteristics of snails younger than one week are different from those of the older ones (Fig. 1). The data for the latter showed a relatively good fit to a straight line. Thus the total weight, in this range, shows the same growth relationship as the one previously established for the shell in a different, although overlapping size range (Nolan and von Brand, 1954). Whether a straight line, with a different slope, can justifiably be drawn for the youngest snails is dubious because of lack of sufficient points. At any rate, the clearly different growth relationship of the youngest snail groups has a biological foundation. The youngest snails did not feed on lettuce, ignoring the lettuce leaves present in each jar. They can not have subsisted solely on reserves carried over from embryonic life, because they

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did increase in weight. It can be presumed that they fed on microorganisms developing in the jars. These groups of snails will hereinafter be designated as prejuveniles.

The prejuvenile groups are followed by the juvenile groups which are defined as beginning with snails older than 8 days, and ending with specimens reaching sexual maturity. The last groups are comprised of mature snails, that is, snails laying eggs. These two groups fed avidly on lettuce.

It must be emphasized that regularities in growth pattern as shown in Fig. 1 can only be demonstrated when the averages of fairly large numbers are used. While the size of the prejuvenile groups was fairly uniform, pronounced variations occurred among the older groups (Table 1). These variations in growth as related to age were definitely not due to an insufficient food supply; food was present always in surplus. The reasons underlying the variations are obscure and will require special analysis, since several possibilities exist; e.g., unequal food utilization (Kleiber, 1947), deficiencies in endocrine functions, and probably others. Such an investigation would be desirable because size is probably one of the factors determining the onset of sexual maturity in the sense that in our experiments snails weighing less than 50 mg never laid eggs. We determined accurately the age and weight reached by 104 specimens at first oviposition. Their average weight was 109 mg with 58.0 and 208.8 as extremes; their average age varied between 47 and 61 days, i.e., the age varied in much narrower limits than the weight. Because of this observation we consider the age factor as more important than did Pereira and Deslandes (1954) who stated that the period of first oviposition was not related to age but to the growth of snail. It should be emphasized, however, that the age figures given above apply only to our set of conditions; under other conditions the minimal age for first oviposition may be lower. Pereira and Deslandes (1954) noted oviposition by 35-day-old Brazilian *Australorbis glabratus*, Maia Penido et al. (1951) and Perlowagora-Szumlewicz (unpublished) by 32-day-old laboratory-reared Brazilian australorbids. In such cases the minimal weight for oviposition, 50 mg, is reached earlier than in our experiment. On the other hand we had in our material 27 specimens that were unusually slow growers, weighing only between

TABLE 1.—OXYGEN CONSUMPTION OF AUSTRALORBIS GLABRATUS OF VARIOUS AGES.*

Group	Number of experiments	Number of snails in individual experiment	Age of snails days	Weight of single snail mg	Mm³ Oxygen consumed in 1 hr.	
					Per single snail	Per mg
Pre-juveniles						
1	4	113–150	0–1	0.07 ± 0.005 (0.06 – 0.08)	0.11 ± 0.010 (0.08 – 0.12)	1.65 ± 0.11 (1.42 – 1.86)
2	3	115	4–5	0.11 ± 0.007 (0.10 – 0.12)	0.12 ± 0.007 (0.11 – 0.13)	1.12 ± 0.02 (1.09 – 1.16)
3	7	46–100	7–8	0.24 ± 0.022 (0.16 – 0.32)	0.15 ± 0.013 (0.11 – 0.20)	0.56 ± 0.05 (0.32 – 0.68)
Juveniles						
1	10	10–18	18–21	3.17 ± 0.70 (1.21 – 8.10)	1.45 ± 0.38 (0.64 – 4.31)	0.45 ± 0.029 (0.30 – 0.58)
2	13	9–18	26–28	10.6 ± 1.4 (5.6 – 22.7)	4.43 ± 0.35 (2.60 – 6.75)	0.45 ± 0.023 (0.30 – 0.58)
3	7	9–10	33–35	20.5 ± 2.4 (13.0 – 30.3)	9.66 ± 0.89 (6.89 – 13.73)	0.48 ± 0.040 (0.32 – 0.61)
4	13	4–11	39–40	43.4 ± 4.4 (19.0 – 72.3)	12.51 ± 1.39 (5.24 – 21.91)	0.29 ± 0.012 (0.20 – 0.34)
Mature snails						
1	60	1–7	47–61	108.7 ± 4.6 (58.0 – 208.8)	29.3 ± 1.40 (15.7 – 62.0)	0.27 ± 0.005 (0.20 – 0.35)

* In this and the following tables the mean values and, in parentheses, the extremes are presented. The figure following the ± sign is the standard error of the mean.

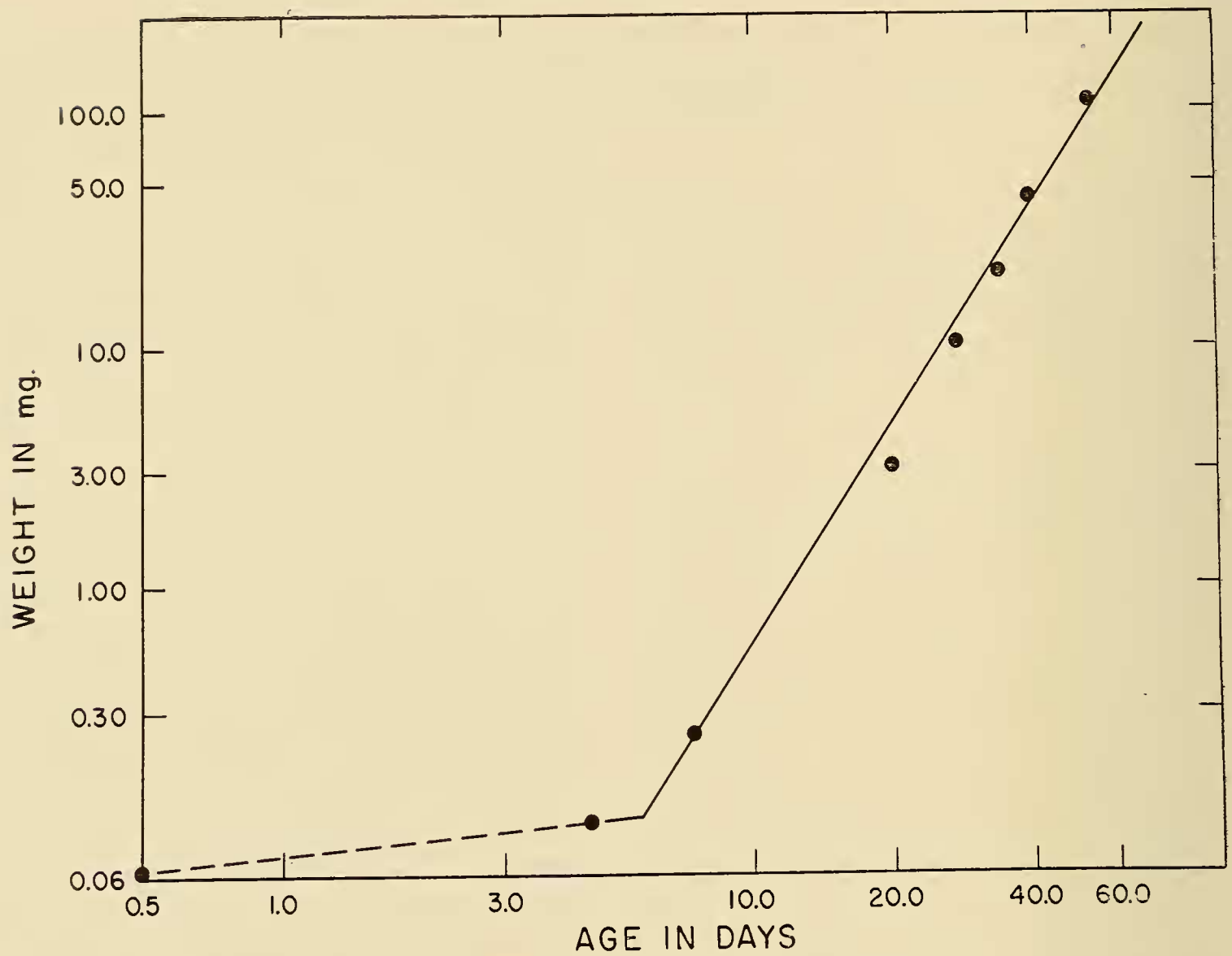

FIG. 1.—Relation between age and weight in growing *Australorbis glabratus*.

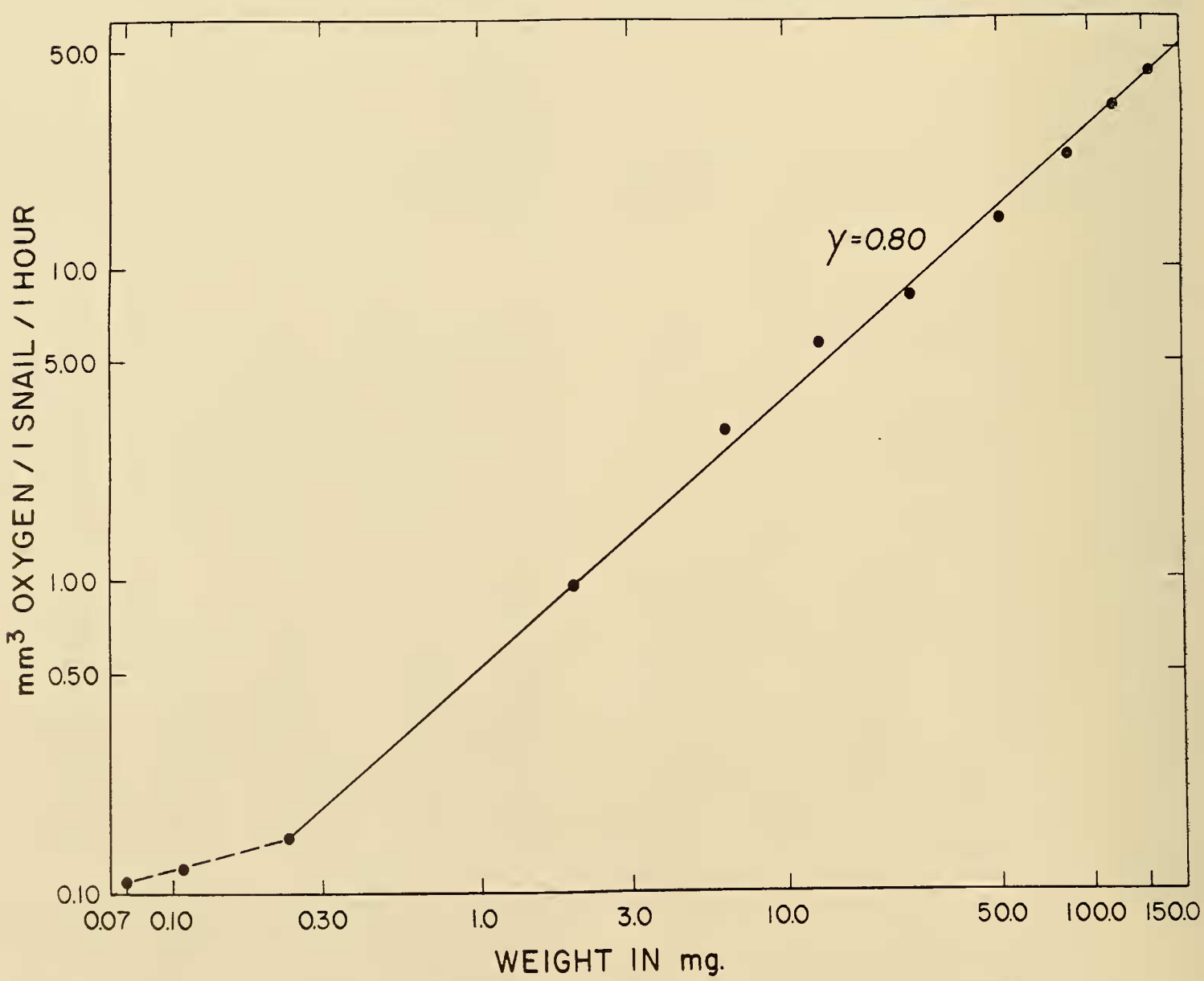

FIG. 2.—Relation between weight and rate of oxygen consumption in growing *Australorbis glabratus*.

24.4 and 44.1 mg when 60 days old. They never laid eggs during the period of observation. It is a matter of conjecture whether they eventually would have reached sexual maturity, or whether they were permanently dwarfed, infantile specimens, as a result, perhaps, of faulty endocrine functions.

Observations of respiratory activity.—As explained above, both the weight and the age of the snails used in the respiration experiments were known; it was therefore possible to correlate the rate of oxygen consumption to both these factors.

As Tables 1 and 2 show, a progressive lowering of the respiratory rates is evident with increasing weight and increasing age, if the rates are calculated on the basis of unit weight. The decrease is greatest in the pre-juvenile groups. Fundamentally, the same trend exists in the other groups, although the decline may be very small if limited size or age differences are considered. The question then arises whether weight or age is primarily responsible for this decline. It is difficult to get incontrovertible evidence on this point, because both factors change in general in the same direction. Our somewhat indirect evidence is as follows: The juvenile groups and the mature group shown in Table 1 had been established purely on the age criterion and therefore each contained both small and large specimens. Each of these groups was now divided into two subgroups (Table 3) one containing the smaller and another one containing the larger snails. In all five groups thus subdivided the respiratory rate of the smaller specimens was higher than that of the larger ones, indicative of a definite trend. Similarly, five groups of the juvenile and mature snails established on the weight criterion (Table 2) contained older and younger specimens. When their average rates were calculated separately, it was found that in two cases the rate of the older specimens was highest, in two other cases that of the younger ones, and in 1 case the rates were equal; that is, a purely random distribution was evident. We are therefore inclined to ascribe to size a more important role in determining metabolic rate than to age, at least in the age range of *Australorbis* studied by us. It is, of course, entirely possible that really senile specimens may behave differently. It should be recalled in this connection that the level of oxidative phosphorylation was reduced in the albumen gland of senile *Lymnaea* (Weinbach, 1956). Planorbid

TABLE 2.—OXYGEN CONSUMPTION OF AUSTRALORBIS GLABRATUS OF VARIOUS SIZES

Group		Number of experiments	Number of snails in individual experiment	Weight of single snail mg	Mm³ oxygen consumed in 1 hour	
					Per single snail	Per mg
Pre-juveniles	1	4	113–150	0.07 ± 0.005 (0.06–0.08)	0.11 ± 0.010 (0.08–0.12)	1.65 ± 0.11 (1.42–1.86)
	2	3	115	0.11 ± 0.007 (0.10–0.12)	0.12 ± 0.007 (0.11–0.13)	1.12 ± 0.02 (1.09–1.16)
	3	7	46–100	0.24 ± 0.022 (0.16–0.32)	0.15 ± 0.013 (0.11–0.20)	0.56 ± 0.05 (0.32–0.68)
Juveniles	1	7	12–15	1.97 ± 0.25 (1.21–3.03)	0.95 ± 0.089 (0.64–1.27)	0.49 ± 0.022 (0.41–0.57)
	2	8	10–18	6.28 ± 0.50 (4.05–8.10)	3.04 ± 0.35 (1.50–4.31)	0.47 ± 0.027 (0.36–0.58)
	3	9	9–15	13.0 ± 0.88 (8.9–16.8)	5.76 ± 0.46 (3.91–8.00)	0.45 ± 0.028 (0.36–0.61)
	4	10	5–11	25.7 ± 1.5 (19.0–33.5)	9.09 ± 0.87 (5.24–13.73)	0.36 ± 0.036 (0.21–0.56)
Mature snails	1*	9	4–5	51.6 ± 4.7 (38.2–60.3)	14.5 ± 0.88 (8.9–16.7)	0.27 ± 0.016 (0.20–0.34)
	2	32	2–7	85.1 ± 1.9 (61.3–105.3)	22.9 ± 0.61 (15.7–29.7)	0.27 ± 0.007 (0.20–0.35)
	3	19	2–3	121.7 ± 1.9 (107.0–137.7)	32.4 ± 1.07 (21.5–38.8)	0.26 ± 0.009 (0.20–0.34)
	4	11	1–3	158.2 ± 6.5 (140.3–208.8)	41.3 ± 3.14 (29.0–62.0)	0.24 ± 0.014 (0.20–0.35)

* This group contains juveniles approaching maturity and mature specimens.

TABLE 3.—ANALYSIS OF RELATIVE IMPORTANCE OF AGE AND SIZE IN DETERMINING THE RESPIRATORY RATE OF AUSTRALORBIS GLABRATUS.*

A. Influence of size (subdivision of age groups shown in Table 1)

Subdivided group	Weight of single snail		Oxygen consumption of heavier snails in per cent of that of lighter ones
	Subdivision a	Subdivision b	
Juvenile 1	1.8	5.2	80
Juvenile 2	7.5	15.3	78
Juvenile 3	14.9	24.8	94
Juvenile 4	28.7	55.9	97
Mature 1	81.6	132.4	96

B. Influence of age (subdivision of weight groups shown in Table 2)

Subdivided group	Age of snails		Oxygen consumption of older snails in percent of that of younger ones
	Subdivision a	Subdivision b	
Juvenile 2	20	27	121
Juvenile 3	27	34	119
Juvenile 4	34	40	64
Mature 1	40	54	100
Mature 2	40	54	93

* For details see text.

snails are reported to reach 2 to 3, occasionally up to 5, years of age[3] (Korschelt, 1922). The 60 days studied by us represent such a small fraction of their life span that a direct age influence can hardly be expected to be demonstrable by the Warburg technique. It is true, however, that we have one observation seemingly in contradiction with the above statement. As mentioned previously, a minority of our snails grew very slowly and did not reach sexual maturity during the time of observation. When the respiratory rate of these older, small specimens was compared with that of younger specimens of approximately equal size, the latter had a distinctly higher rate (Table 4). However, the small, but older, snails must probably be regarded as pathologically retarded specimens. It seems more likely that whatever factor (e.g., endocrine or other) was responsible for their retardation rather than age proper, was responsible for the lowered rate. It certainly would seem desirable to exclude such snails from studies concerned with the normal metabolism. Since they look externally normal, they can be weeded out only when the age is accurately known. In experiments where the greatest obtainable uniformity of material is desirable, both the age and size factors should be considered.

TABLE 4.—OXYGEN CONSUMPTION OF AUSTRALORBIS GLABRATUS OF APPROXIMATELY EQUAL SIZE, BUT DIFFERENT AGE

Number of experiments	Total number of snails	Age of snails days	Weight of single snail mg	Mm³ Oxygen consumed in one hour	
				Per snail single	Per mg
8	77	27–34	21.3 ± 1.9 (15.3–30.3)	9.0 ± 0.95 (6.0–13.7)	0.43 ± 0.033 (0.30–0.56)
5	35	40–41	26.8 ± 2.8 (19.0–33.5)	7.7 ± 1.02 (5.2–10.5)	0.28 ± 0.025 (0.21–0.33)
4	21	61–63	26.0 ± 3.8 (17.4–32.8)	6.7 ± 0.78 (4.6–7.8)	0.26 ± 0.012 (0.24–0.29)

It has been shown previously (von Brand et al., 1948) that a fairly good agreement existed in *Australorbis* between respiratory rate and relative surface, an agreement much better than between weight and rate. The materials used then and now are not completely comparable, because then the age factor was not considered and it is conceivable that some retarded snails had been included; furthermore the experiments of 1948 were done at 30°C, the present ones at 28°C. It is nevertheless of some interest to compare briefly both series in regard to the overlapping size groups. Table 5 shows an excellent agreement between both series which is rather surprising in view of the above differences and the fact that the strain was then newly established in the laboratory but by now is an old laboratory strain. It seems evident that in the size range discussed the oxygen consumption follows more closely, though not completely, the relative surface ($W^{2/3}$) rather than weight.

TABLE 5.—COMPARISON OF 1948 DATA (VON BRAND ET AL.) AND PRESENT DATA ON RESPIRATORY RATE OF AUSTRALORBIS GLABRATUS. (The 1948 data are in italics.)

Weight of individual snail, mg	Mm³ Oxygen 1 snail 1 hour	Relative surface	Ratios of—		
			Weight	Oxygen	Relative surface
13.0	5.8	5.5	1.0	1.0	1.0
14	*5.9*	*5.8*	*1.1*	*1.0*	*1.1*
25.7	9.09	8.7	2.0	1.6	1.6
51.6	14.48	13.9	4.0	2.4	2.5
66	*16.8*	*16.3*	*5.1*	*2.9*	*3.0*
85.1	22.85	19.4	6.5	3.9	3.5
121.7	32.35	24.6	9.3	5.6	4.5
153	*30.3*	*28.6*	*11.7*	*5.2*	*5.2*
158.2	41.3	29.3	12.2	7.1	5.3

When the smaller size groups were included into the above calculation, the deviations from the expected values became more marked. This suggested that the relationship $W^{2/3}$ was not entirely satisfactory to describe the weight/oxygen relations. We therefore analyzed the entire size range used in the present study by the allometric procedure recommended by von Bertalanffy (1951). The respiratory figures are plotted against weight on a double logarithmic scale, resulting usually in a straight line. Calculation of the tangent of the angle formed with the abscissa yields the power to which the body weight must be raised to describe the relationship between body weight and respiration. Upon applying this procedure to our data, it became evident that the pre-juvenile groups behaved differently than the remaining groups (Fig. 2). This is not surprising since in practically all animals studied so far, the relationship between size and oxygen consumption differs in the smallest specimens from that prevailing after the organisms have undergone a certain growth and

[3] The exact life span of *A. glabratus* is unknown. According to unpublished observations by Perlowagora-Szumlewicz only a small percentage survives in captivity longer than 24 months.

possibly again after they reach near maximum size (Zeuthen, 1953). It is evident from Fig. 2 that an identical relationship exists for the juvenile and adult groups; it does not follow weight ($y = 1.0$) nor surface as calculated by the formula $W^{2/3}$ ($y = 0.666$), but is actually intermediate. The exponent found by us ($y = 0.80$) is quite similar to that found by von Bertalanffy (1951) for *Planorbis* ($y = 0.75$). It must be recognized that one factor exists which introduces a certain amount of uncertainty into the above calculations; the possibility that the metabolically more or less inert shell varies significantly in weight in snails of different size. Indications to this effect have been reported for the larger size range of *Australorbis* (Nolan and von Brand, 1954), but the relation between shell weight and total weight for the smaller size groups has not been established for our strain.[4] It is clear that any error introduced by this factor is reflected in all calculations but it becomes smaller as the exponent to which the weight is raised becomes smaller.

SUMMARY

1. The growth characteristics of *Australorbis* differ during the first week after hatching from those of older specimens.

2. Both size and age seem responsible for onset of sexual maturity.

3. If the whole size range from freshly hatched to sexually mature snails is considered, the respiratory rate decreases materially with increasing size or age if the rate is referred to unit weight, although the decline may be quite small within limited size or age ranges.

4. Size has probably a greater importance than age in determining metabolic rate, at least in the age range studied.

5. While in larger snails a fairly good correlation exists between relative surface and respiratory rate, inclusion of the smaller juveniles leads to discrepancies, indicating that the formula $W^{2/3}$ is not entirely adequate. The exponent 0.80 fits the size range studied much better.

[4] Perlowagora-Szumlewicz (unpublished) considers the age factor as more important in determining the relative shell weight than the size factor.

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To treat your facts with imagination is one thing; to imagine your facts is quite another.—JOHN BURROUGHS.